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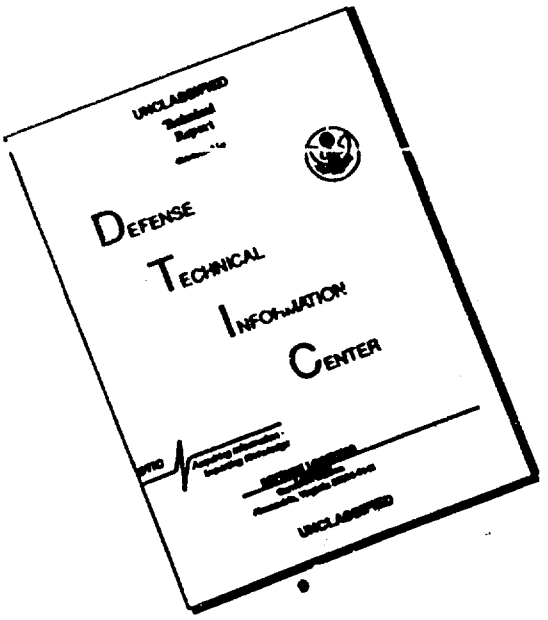

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THE FLUORESCENT-SEROLOGICAL METHOD IN THE DIAGNOSIS OF TYPHOID AND A AND B PARATYPHOID FEVER

[Following is the translation of an article by L.V. Mirolyubova and G.S. Dvurechinskaya (Epidemiology and Microbiology Institute imeni Gamaleya of the Academy of Medical Sciences USSR and the infectious disease clinic of the Second Moscow Medical Institute imeni Pirogov) in the Russian-language publication Zhurnal Mikrobiologii, Epidemiologii, i Immunologii (Journal of Microbiology, Epidemiology, and Immunology), Vol XXXIII, No 10, Moscow, 1962, pages 3-7.]

The earliest and most reliable confirmation of the clinical diagnosis of typhoid and paratyphoid, is, as we know, the detection of the causative agents in the blood. But the classical investigative technique for the isolation of the hemoculture requires several days, so that some researchers have made highly justified attempts to develop more rapid diagnostic methods. In this regard, one of the most promising ones is the luminescent-serological technique.

The literature contains only individual references on the detection of bacteria in the blood of patients by the method of luminescent antibodies. Thus, Gol'din and Amosenkova (1960) cite data on the detection of Burnett rickettsia in blood smears from Q fever patients. The authors note here that reliable identification of the causative agent is possible only with a count of not less than 2 million rickettsial cells per ml of blood in the case of the luminescent-serological method. Trount (1959) used luminescent antibodies in the examination of blood and spinal fluid for the presence of typhoid bacteria.

In our studies for the purpose of detecting typhoid and paratyphoid A and B bacteria in the blood of patients, we used both the direct and indirect luminescent-serological method.

Luminescent serums were obtained from the globulin fractions of antityphoid and A and B antiparatyphoid fever serums which were labelled by the Coons method with fluorescein isocyanate prepared at the Chemical Reagents Institute by a group of researchers headed by G.I. Mikhaylov. The initial serums were dilute native agglutinating and special adsorbed serums prepared by the Moscow Epidemiology and Microbiology Institute. The indirect method involved the use of monoreceptor salmonella rabbit O-serums (II, IV, V, IX, and Vi receptors) obtained from the Leningrad Vaccine and Serum Institute. The antiglobulin (antirabbit) asinine serum was prepared in the immunology and oncology department of the Epidemiology and Microbiology Institute imeni Gamaleya of the Academy of Medical Sciences USSR.

Preliminary studies carried out on pure cultures of various types of bacteria evidenced the high specificity of the luminescent serums.

On the basis of the possibilities of the luminescent-serological method and the pathogenesis of typhoid, we assumed that bacteria could be detected directly in blood smears. However, typhoid in a number of cases is accompanied by such an insignificant quantitative bacteremia that the accumulation and concentration of bacteria is required for their detection. For this reason, we developed a special procedure consisting in the cultivation of the bacteria in the blood in a 5% bile bouillon for 18 hours, followed by 10-15 minutes of centrifuge at 10,000 rev/min. The residue was smeared on slides, fixed with Carnois mixture or ethyl alcohol and treated with luminescent serums. Preparation of the slides took not more than an hour. Microscopic examination of the slides was carried out in blue incident light (SVDSh-250-3 illuminator lamp, SZS-7, SS-8, SS-4, ZhS-18 light filters) with a magnification of 360-500 X (90/1.25 or 100/1.30 objectives, oil immersion, and 4 X or 5 X oculars).

We considered an analysis as positive if the slides revealed even single cells with the characteristic peripheral glow.

At the same time, we carried out the isolation of the hemoculture by the classical method, taking into account all of the blood tests previously administered over the observation period.

Blood samples for luminescent-serological testing were taken from patients entering the clinic with possible typhoid-paratyphoid complaints directly in the receiving department or on the first or second day after admission to the hospital. In nine patients blood tests were made during the first week of illness, and in the remaining patients -- after the 15th day of illness. For 10 patients, blood tests were repeated several days after entry into the clinic.

We examined a total of 126 patients and performed 136 blood tests. Typhoid and paratyphoid diagnoses were based on a sufficiently characteristic clinical picture of the disease (fever, typhous status, rash, bradycardia, enlargement of liver and spleen, typical appearance of tongue, etc.). 47 patients were diagnosed as having typhoid, 7 -- type A paratyphoid, and 17 -- type B paratyphoid. The illness took grave form in 15 patients; in 43 it was of medium severity. A bacteriological confirmation of the diagnosis was obtained for 28 patients (in 25 of them by the isolation of the hemoculture); serological confirmation was obtained for 20 patients (according to a positive Vidal reaction).

The direct luminescent-serological method was used to detect bacteria in the blood of 41 patients, while the hemoculture was isolated in 25. The coincidence of positive results was observed in 24 patients. The increase in positive results was due mainly to the method of luminescent antiboides and only in one case [see note] to bacteriological examination. ([Note:] It should be noted that the hemoculture in this case was isolated from a blood sample taken from the patient on the previous day. In the blood sample provided for luminescent-serological study, negative results were obtained both serologically and bacteriologically.) (Tables 1 and 2).

The increase in positive cases due to the results of luminescent microscopy can be explained by the microbiological fact that microorganisms with reduced growth activity weakened by any external factors, grow weakly in a liquid nutritive medium and do not form colonies upon transplantation into a solid medium.

Data from control studies confirmed the specificity of the positive results obtained for patients with a clinical diagnosis of typhoid or A and B paratyphoid (experiments with pure cultures. the presence of specific luminescence only in smears treated with one serum, etc.).

The indirect luminescent-serological method was used for the simultaneous examination of blood from 59 patients, including typhoid and A and B paratyphoid patients (of these, 14 revealed a positive result). To detect A paratyphoid bacteria, we employed a monoreceptor salmonella O-serum (receptor II), for the detection of of B paratyphoid bacteria -- a mixture of serums (receptors IV and V), and for the detection of typhoid bacteria -- a mixture of the IX and Vi receptors.

The results obtained by the indirect method largely coincided with the results of the direct luminescent-serological method (Table 3).

Only in the analyses for 3 patients whose blood revealed typhoid bacteria in the direct method, did the indirect method yield a positive results with two samples simul-

taneously -- both in smears treated with a mixture of monoreceptor salmonella O-serums (IX and Vi) and smears treated with a serum containing receptor II. The hemocultures isolated for two of these patients behaved biochemically as typical typhoid bacteria. The agglutination reaction with these cultures was observed upon the solution of the agglutinating anti-typhoid serum 25,600 times. The cultures were not agglutinated by special adsorbed anti-paratyphoid A and B serums. But definite agglutination was observed with monoreceptor salmonella O-serums (receptors II, IX, and Vi). The O-antigen receptor II contained in these typhoid strains was also detected by the indirect luminescent-serological method. Thus, there were no disparities between the results of the indirect luminescent-serological method and bacteriological tests.

Table 1

Results of (Direct) Luminescent-Serological and Bacteriological Tests of Patients' Blood

Diagnosis	Number of test subjects	Number of test subjects with positive result	Including		
			Coincidence of results with the two methods	Only with luminescent serological method	Only with hemoculture isolation
Typhoid A	47	25	14	10	1
" B	7	7	5	2	-
Paratyphoid	17	10	5	5	-
Total	71	42	24	17	1

In the study of blood from 55 patients with various febrile ailments (infectious mononucleosis, infectious erythema, rheumatism, pneumonia, food toxicoinfectious, etc.), the bacteriological and (direct) luminescent methods did not give positive results in a single case. Slides prepared from the blood of 4 patients and treated with luminescent serums revealed weak, poorly contrasting rod-like cells which were quite distinct from the peripherally bright specific agents. Unfortunately, the bacteriological method was not

successful in isolating the cultures from these patients.

Table 2

Results of Bacteriological and Luminescent-Serological Studies Depending on the Time of Blood Sampling for Typhoid and A and B Paratyphoid Patients

Time after start of ailment	Number of test subjects	Number of cases of culture isolation	Positive result in luminescent-serological test
1-7th day	9	6	9
8-14th day	36	15	22
15th day and later	26	4	10
Total	71	25	41

Table 3

Results of Studies of Patients' Blood by Indirect Luminescent-Serological Method (Monoreceptor Rabbit Salmonella O-serums + Luminescent Anti-Rabbit Serum)

Diagnosis	Number of test subjects	Positive results				
		By direct method	with monoreceptor O serums			
			II	IV+V	IX+Vi	total
Typhoid	17	9	3	-	9	9*
Paratyphoid A	2	2	2	-	-	2
" B	5	3	-	3	-	3
Various febrile diseases	35	-	-	-	-	-
Total	59	14	5	3	9	14*

[* The discrepancy in these totals is in the original -- Trans.]

Thus, our studies have shown the possibility of using luminescent antibodies for the acceleration of the early laboratory diagnosis of typhoid and A and B paratyphoid (for the purpose of detection of bacteria in the blood).

Conclusions

1. The luminescent-serological method is a promising one in blood testing and can be used for the accelerated laboratory diagnosis of typhoid and A and B paratyphoid.
2. The luminescent-serological method, with cultivation and concentration of the initial material (blood) is more sensitive than the hemoculture isolation technique.

References

Gol'din and Amosenkova in the book The Problem of Rickettsiosis. Abstracts of Reports. Krasnodar, 1960, page 56.
Traunt (1959) citing Coons in Schweiz Z. allg. Path., 1959, Vol 22, page 700.

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